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Hippo signalling in the liver: role in development, regeneration and disease

Jacquelyn O. Russell[1,2] *and Fernando D. Camargo*[1,2]✉

Abstract | The Hippo signalling pathway has emerged as a major player in many aspects of liver biology, such as development, cell fate determination, homeostatic function and regeneration from injury. The regulation of Hippo signalling is complex, with activation of the pathway by diverse upstream inputs including signals from cellular adhesion, mechanotransduction and crosstalk with other signalling pathways. Pathological activation of the downstream transcriptional co-activators yes-associated protein 1 (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ, encoded by *WWTR1*), which are negatively regulated by Hippo signalling, has been implicated in multiple aspects of chronic liver disease, such as the development of liver fibrosis and tumorigenesis. Thus, development of pharmacological inhibitors of YAP–TAZ signalling has been an area of great interest. In this Review, we summarize the diverse roles of Hippo signalling in liver biology and highlight areas where outstanding questions remain to be investigated. Greater understanding of the mechanisms of Hippo signalling in liver function should help facilitate the development of novel therapies for the treatment of liver disease.

Chronic liver disease (CLD) is a global epidemic, with deaths from CLD continuing to rise annually[1]. Cirrhosis and liver cancer together account for approximately two million deaths globally each year[1], despite the fact that the liver has immense regenerative potential. The differentiated epithelial cells of the liver, hepatocytes and cholangiocytes (also known as biliary epithelial cells), are capable of exiting quiescence and proliferating to repair hepatic damage[2]. It is theorized that repeated bouts of hepatic damage, such as from alcoholic hepatitis, viral hepatitis, nonalcoholic fatty liver disease, cholangiopathies, etc., can over time exhaust the liver's regenerative potential, leading to the progression of fibrosis to cirrhosis and potentiating the development of liver cancer[3]. Currently, therapies that prevent or reverse progression of CLD are extremely limited, and the only curative treatment for end-stage liver disease is liver transplantation. However, the demand for donor organs far exceeds the supply[1]. Therefore, there is a vast unmet clinical need for effective therapies for CLD.

Over the past decade, it has become apparent that the Hippo signalling pathway has major roles in many aspects of hepatic function, including regulation of liver development, homeostasis, regeneration and cancer. First identified as a regulator of tissue growth in *Drosophila*[4,5], the corresponding mammalian pathway was quickly identified and found to regulate organ size, as inhibition of Hippo signalling led to a dramatic overgrowth phenotype in the liver[6,7]. Since then, Hippo signalling has been found to regulate a variety of key cellular processes such as cell fate, metabolism and tumorigenesis, and the immune system. Much research has been devoted to identifying the complex upstream regulators of this pathway, which has demonstrated that Hippo signalling is modulated by a vast array of biochemical and biophysical inputs. In this Review, we provide an overview of the role of Hippo signalling in many aspects of liver function and discuss the potential development of novel therapies for CLD targeting components of the Hippo signalling pathway.

Canonical Hippo signalling pathway

This conserved pathway was first identified in *Drosophila*, where deletion of the serine–threonine protein kinase hippo led to a dramatic overgrowth phenotype[4]. The corresponding mammalian proteins (FIG. 1) are the serine–threonine protein kinases MST1 (also known as STK4) and MST2 (also known as STK3)[8,9], which form a complex with the scaffolding protein salvador homologue 1 (SAV1; also known as WW45)[10]. MST1 and MST2 phosphorylate and activate serine–threonine protein kinase LATS1 and LATS2 (homologues to the *Drosophila* protein Warts)[4,11]. Merlin (also known as NF2) facilitates the activation of LATS1 or LATS2 by

[1]*Stem Cell Program, Boston Children's Hospital, Boston, MA, USA.*

[2]*Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA.*

✉*e-mail: Fernando.Camargo@childrens.harvard.edu*

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Key points

- During embryonic liver development, yes-associated protein 1 (YAP) activity in hepatoblasts suppresses hepatocyte differentiation and is essential for proper cholangiocyte maturation and formation of the intrahepatic biliary network.
- During adult liver homeostasis, YAP activity is largely dispensable in hepatocytes but is required for the maintenance of mature cholangiocytes through its protective effects against exposure to hydrophobic bile acids.
- YAP is an important regulator of liver cell fate owing to its ability to promote hepatocyte-to-cholangiocyte transdifferentiation, and its activity in cholangiocytes drives the ductular reaction during chronic liver injury.
- YAP–transcriptional co-activator with PDZ-binding motif (TAZ) activity in hepatocytes, hepatic stellate cells, Kupffer cells and liver sinusoidal endothelial cells promotes inflammation and fibrosis in multiple models of nonalcoholic steatohepatitis and chemical-induced liver fibrosis.
- YAP is a potent oncogene and high YAP activity is frequently observed in hepatocellular carcinoma, hepatoblastoma and cholangiocarcinoma, where it promotes tumour cell proliferation, drug resistance and potentially immune evasion.
- Pharmacological targeting of YAP–TAZ holds great potential, either through inhibition of YAP–TAZ activity for anti-cancer or anti-fibrotic therapies or through promotion of YAP–TAZ activity to enhance liver regeneration.

MST1 or MST2 through recruitment of these proteins to the plasma membrane[12,13]. LATS1 and LATS2, together with the regulatory proteins MOB kinase activators 1A (MOB1A) and MOB1B, phosphorylate yes-associated protein 1 (YAP; homologue to the *Drosophila* protein Yorkie[14]) at multiple serine residues[15], including S127 in the human protein[6] or S112 in the mouse protein. Transcriptional co-activator with PDZ-binding motif (TAZ), a paralogue of YAP, is also phosphorylated by this complex[16]. The phosphorylation of YAP–TAZ causes the scaffolding protein 14-3-3 to bind to and sequester them in the cytoplasm and eventually target them for proteasomal degradation via F-box/WD repeat-containing protein 1A (encoded by *BTRC*; also known as β-TRCP)[15,17] (FIG. 1a). When YAP and TAZ are hypophosphorylated, they can translocate to the nucleus and interact with the TEAD family of transcription factors to mediate target gene expression[18,19] (FIG. 1b). Thus, the canonical Hippo signalling pathway functions to suppress the activity of YAP–TAZ.

Upstream regulators of Hippo signalling

Although Hippo signalling regulates many aspects of hepatic function, much remains unknown about the upstream signals that regulate the activity of the pathway. In this section, we summarize the currently known upstream regulators of Hippo signalling, but more research is required to fully elucidate these mechanisms.

Cellular polarity and adhesion. A major function of Hippo signalling is to restrict cell proliferation, such as during contact inhibition. During in vitro culture, diverse cell types grown at low density exhibit nuclear YAP, whereas YAP is predominantly cytoplasmic in high-density cell culture[17,20,21]. Mechanistically, this regulation of Hippo signalling is in part mediated by protein complexes involved in junction formation and cell polarity. Assembly of the tight junction-associated Crumbs complex in multiple types of high-density epithelial cells was found to increase YAP–TAZ phosphorylation, leading to their cytoplasmic sequestration[20]. Scribble, a protein regulating apical–basal polarity, is also required for Hippo activity in mammalian cells by promoting the formation of the membrane complex of MST, LATS and TAZ proteins[22]. The tumour suppressor liver kinase B1 (LKB1) was found to promote the proper localization of Scribble to this membrane complex through activation of proteinase-activated receptor family proteins, thus activating Hippo signalling[23] (FIG. 1a), although other groups have reported LKB1 inhibition of YAP in a LATS1/LATS2-independent manner in HeLa cells[24]. Importantly, liver-specific deletion of LKB1 results in increased hepatocyte proliferation and hepatomegaly. This phenotype was rescued by co-deletion of YAP[23], demonstrating a role for cell polarity complexes in regulating Hippo signalling in the liver.

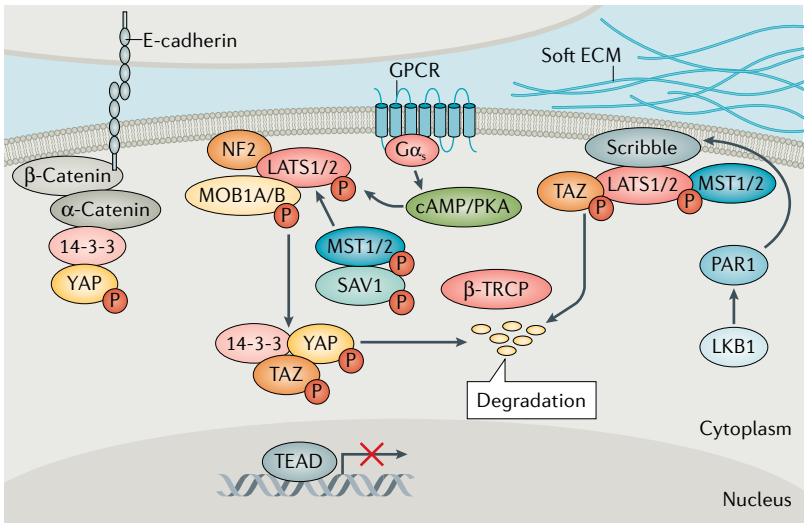
Components of the adherens and tight junctions have themselves also been shown to regulate Hippo signalling. In human keratinocytes the adherens junction protein α-catenin inhibits YAP activity by forming a cell membrane complex with S127-phosphorylated YAP and 14-3-3, which also reduces the accessibility of YAP to protein phosphatase 2A[21]. Indeed, liver-specific knockdown of α-catenin prior to two-thirds partial hepatectomy in mice increased YAP activation and hepatocyte proliferation, and triggered hepatomegaly, disorganization of the actin cytoskeleton, disrupted tight junctions and dilated bile canaliculi[25]. In human breast cancer cell lines, E-cadherin promoted contact inhibition through regulation of Hippo signalling components, and depletion of E-cadherin, β-catenin or α-catenin promoted YAP nuclear localization[26]. In canine kidney epithelial cells, mechanical strain triggered rapid YAP nuclear translocation and cell cycle re-entry, and E-cadherin homotypic cell–cell adhesion was found to maintain quiescence and prevent nuclear accumulation of YAP[27]. Interestingly, YAP can also regulate junction formation, as overexpression of transgenic YAP in mouse hepatocytes antagonized E-cadherin-mediated adherens junction formation and inhibited hepatocyte cell–cell adhesion in vitro[28]. These data suggest that crosstalk between cell polarity and junctional complexes is a key regulator of Hippo–YAP signalling.

Biomechanical regulation. Mechanotransduction has also been identified as an important regulator of Hippo–YAP signalling and it can operate in both a Hippo component-dependent and a Hippo component-independent manner (reviewed previously[29]). YAP–TAZ activity is governed by both stiffness and cell geometry: YAP–TAZ underwent nuclear translocation and increased transcriptional activity in multiple human cell lines grown in vitro on extracellular matrix (ECM) with high stiffness or during cell spreading[30,31]. This activation required the formation of stress fibres and the activity of the small GTPase Rho independently of the Hippo cascade[30,31] (FIG. 1b). However, other groups have reported that the regulation of YAP by the actin cytoskeleton requires Hippo signalling, as actin cytoskeleton disruption-induced YAP inhibition required LATS1 and LATS2 activity[32,33]. Importantly, a 2019 study demonstrated that mechanical tension and formation of stress

fibres triggers YAP activation in cultured primary mouse hepatocytes, triggering hepatocyte dedifferentiation and loss of hepatocyte function[34]. Restricting cell spreading, deletion of *Yap1* specifically from hepatocytes, or treatment with a cocktail of drugs targeting actin polymerization and actomyosin contraction maintained hepatocyte function during long-term culture[34], demonstrating the crucial role of mechanotransduction in the regulation of YAP activity in hepatocytes.

Additionally, YAP–TAZ have been shown to activate Hippo signalling in a negative feedback loop through increased transcription and activation of LATS1 and LATS2, NF2 and the angiomotin (AMOT) protein family[35–37]. The AMOT protein family consists of angiomotin (encoded by *AMOT*), AMOTL1 and AMOTL2, which bind to F-actin. Disruption of the actin cytoskeleton frees AMOT proteins to bind to and sequester YAP–TAZ in the cytoplasm[35,38]. Additionally, it was shown through an in vitro kinase assay that LATS2 can phosphorylate residues in the actin-binding domain of angiomotin[38]. Once freed from F-actin, angiomotin binds to YAP and promotes its cytoplasmic localization in multiple human cell lines[38]. However, angiomotin has also been found to promote YAP activity. NF2 is a known tumour suppressor, and mice with liver-specific deletion of NF2 develop liver cancer[39,40]. Liver-specific dual deletion of *Nf2* and *Amot* reduces tumorigenesis in mice, and in NF2-deficient cells angiomotin binds to YAP and promotes its nuclear translocation and transcription of target genes[40]. Thus, in the context of the mouse liver, angiomotin functions to promote YAP activity[40].

a Hippo signalling on



b Hippo signalling off

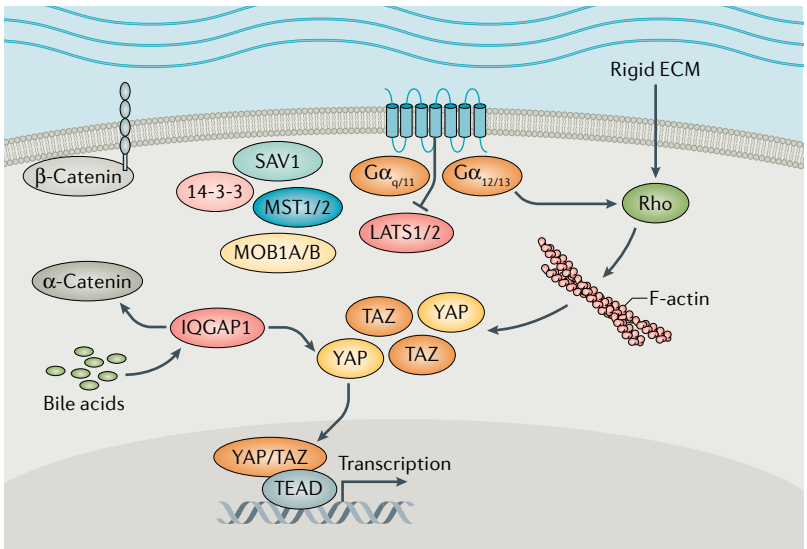


Fig. 1 | **Hippo signalling pathway. a** | The Hippo signalling pathway functions to repress activity of the transcriptional co-activators yes-associated protein 1 (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ). The kinases MST1 (also known as STK4) and MST2 (also known as STK3) form a complex with the scaffolding protein salvador homologue 1 (SAV1) and phosphorylate the kinases LATS1 and LATS2 in a manner facilitated by NF2 (also known as Merlin). Together with the regulatory proteins MOB kinase activators 1A (MOB1A) and MOB1B, LATS1/LATS2 phosphorylate YAP–TAZ, which are bound by 14-3-3 and targeted for proteasomal degradation by F-box/WD repeat-containing protein 1A (also known as β-TRCP). Other inputs such as liver kinase B1 (LKB1) or G protein-coupled receptor (GPCR) signalling or contact inhibition through adherens junctions can suppress YAP–TAZ activity. **b** | Multiple upstream inputs such as stiff extracellular matrix (ECM), low cell density, bile acid exposure, GPCR signalling and increased actomyosin tension can inhibit Hippo signalling. Non-phosphorylated YAP and TAZ are no longer targeted for degradation and translocate to the nucleus where they interact with TEAD transcription factors to promote target gene expression. IQGAP1, Ras GTPase-activating-like protein 1.

Input from other signalling pathways. In addition to its regulation by biomechanical cues, many other inputs are known to regulate Hippo signalling. For example, Hippo signalling is modulated by diverse aspects of cell function, such as responses to oxidative stress[41] and many aspects of cellular metabolism[42]. G protein-coupled receptor (GPCR) signalling can modulate Hippo signalling; lysophosphatidic acid or sphingosine 1-phosphate-activated $G\alpha_{12/13}$-coupled or $G\alpha_{q/11}$-coupled receptors, which in turn inhibit LATS1 and LATS2 and increases YAP activation in multiple human cell lines[43] (FIG. 1b). Subsequent studies have shown that non-canonical WNT ligands (WNT3A, WNT5A and WNT5B)[44] and thrombin[45] can signal through $G\alpha_{12/13}$-coupled receptors to activate Rho GTPase, which in turn inhibits LATS1 and LATS2 and promotes YAP–TAZ activation. However, the role of these pathways in physiological activation of YAP activity remains to be determined.

Signalling pathways involving tyrosine kinase receptors such as epidermal growth factor receptor (EGFR)[46], fibroblast growth factor receptor (FGFR)[47] and insulin signalling[48] have been shown to crosstalk with Hippo signalling. These pathways are especially relevant in the context of liver cancer and are discussed in more detail later in this Review. The non-receptor tyrosine kinases SRC and focal adhesion kinase (FAK) can regulate Hippo signalling through multiple mechanisms. In mouse keratinocytes, SRC has been shown to activate YAP through direct phosphorylation of residues Y341, Y357 and Y394 (REF.[49]). SRC has additionally been shown to phosphorylate and inhibit LATS1 (REF.[50]). Nemo-like kinase has been found to phosphorylate YAP on S128 in response to osmotic stress, which increases YAP activity through reduced proteasomal degradation[51,52]. YAP activity is also regulated by lysine methyltransferases. In human embryonic kidney cells, methylation of YAP K494

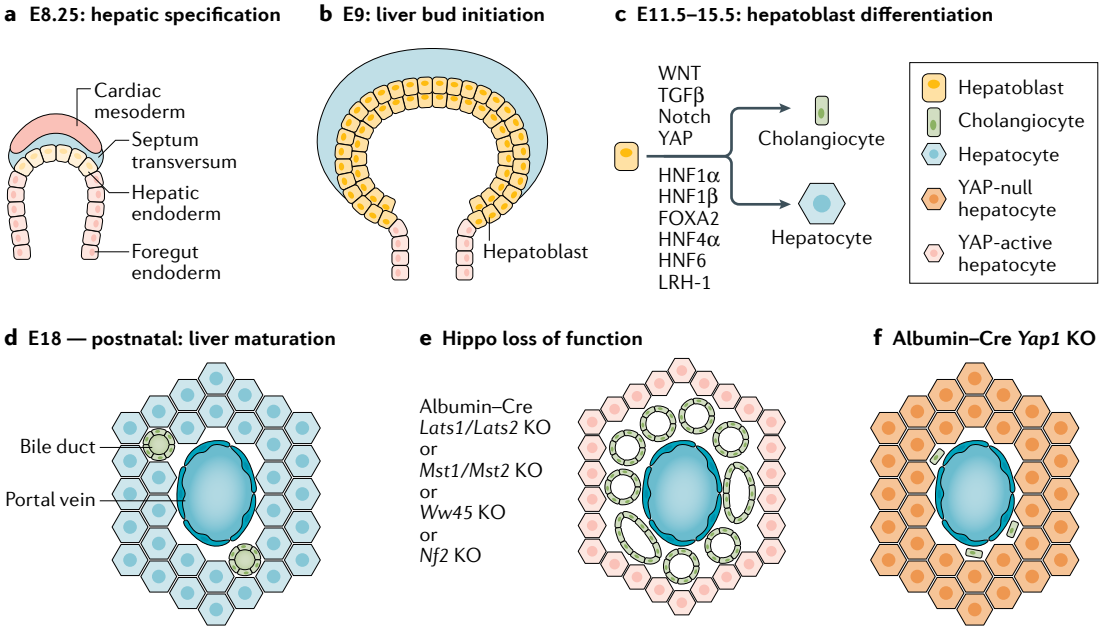


Fig. 2 | **Role of Hippo signalling in liver development. a** | During mouse embryonic development, hepatic specification occurs at embryonic day 8.25 (E8.25). Interactions between the foregut endoderm, the septum transversum and the cardiac mesoderm are required for the specification of hepatic endoderm. **b** | Hepatoblasts, the embryonic precursors to hepatocytes and cholangiocytes, first appear in the liver bud at E9. **c** | Hepatoblast differentiation to hepatocytes or cholangiocytes occurs between E11.5 and E15.5. Cholangiocyte differentiation requires WNT, transforming growth factor-β (TGFβ) and Notch signalling, while hepatocyte differentiation requires a regulatory network of transcription factors. **d** | Liver maturation continues after birth to form the mature hepatic architecture. **e** | Hyperactivation of yes-associated protein 1 (YAP) through deletion of upstream Hippo components via albumin–Cre results in failure to form mature hepatocytes and expansion of immature biliary cells. **f** | Deletion of *Yap1* via albumin–Cre impairs bile duct formation and reduces hepatocyte viability. FOXA2, forkhead box A2; HNF, hepatocyte nuclear factor; KO, knockout; LRH-1, liver receptor homologue 1.

by SET7 inhibits YAP activity by promoting cytoplasmic retention[53], while methylation of YAP K342 by SET1A promotes YAP activity through increased nuclear accumulation in multiple human cell lines[54]. Nuclear receptors have also been shown to regulate YAP activity. In vivo stimulation of the pregnane X receptor (PXR) in mice induces hepatomegaly, as PXR binds to YAP and promotes its nuclear translocation and subsequent hepatocyte proliferation[55]. The nuclear receptors farnesoid X receptor and small heterodimer partner regulate bile acid levels within the liver, and bile acids have been shown to regulate YAP activity. Dietary bile acid overload in mice induced elevated bile acid levels in the liver and activated YAP in hepatocytes through increased levels of the scaffolding protein Ras GTPase-activating-like protein IQGAP1 (REF.[56]). Interestingly, overexpression of IQGAP1 inhibits cell–cell adhesion by promoting dissociation of α-catenin from E-cadherin and β-catenin in mouse L cells[57], suggesting a link between bile acid regulation of YAP and regulation of adherens junctions. Collectively, these studies demonstrate the complex regulation of YAP activity by myriad upstream inputs.

Downstream targets of YAP–TAZ

YAP–TAZ promote the activation of a wide variety of signalling pathways in a cell type-dependent and context-dependent manner. As is discussed in more detail in the following sections, YAP and TAZ are known to regulate Notch signalling[58], anti-apoptotic pathways[6], transforming growth factor-β (TGFβ) signalling[59], actin polymerization[60], Hedgehog signalling[61], mammalian target of rapamycin complex 1 (mTORC1) activity[62], nucleotide biosynthesis[63], AKT signalling[64] and more.

Hippo signalling in liver development

During mouse embryonic development, the liver is derived from the foregut endoderm. The activity of the transcription factors hepatocyte nuclear factor 1β (HNF1β), forkhead box A1 (FOXA1), FOXA2 and GATA-binding protein 4 (GATA4) are crucial for hepatic specification, which occurs at approximately embryonic day 8.25 (E8.25) within the foregut endoderm[65] (FIG. 2a). At E9.0, the liver diverticulum forms from the ventral domain of the foregut endoderm and subsequently develops into the liver bud containing hepatoblasts, which are the bipotent embryonic precursors to both hepatocytes and cholangiocytes (FIG. 2b). As the mechanisms underlying hepatoblast differentiation are complex, for a more in-depth review of this topic we direct the reader to REFS[65,66]. In general, activation of TGFβ, Notch, WNT–β-catenin and other signalling pathways promote a cholangiocyte fate within hepatoblasts[66], whereas hepatocyte fate depends on the activity of a core regulatory network of transcription factors consisting of HNF1α, HNF1β, FOXA2, HNF4α, HNF6 and liver receptor homologue 1 (REF.[67]) (FIG. 2c). Interestingly, single-cell RNA sequencing (scRNA-seq) analysis of

isolated hepatoblasts from multiple time points during fetal mouse liver development showed that hepatoblast differentiation to hepatocytes is the default cell fate, with differentiation occurring from E13.5 to E15.5. By contrast, cholangiocytes were specified as early as E11.5 but more prominently by E13.5, with cholangiocyte maturation being driven by activation of mitogen-activated protein kinase (MAPK) signalling[68]. By E18, cholangiocytes have begun to organize into tubules, which eventually form mature bile ducts as cholangiocyte maturation continues in the postnatal period[66] (FIG. 2d).

Although the mechanisms of hepatoblast specification to hepatocytes or cholangiocytes remain incompletely understood, several studies have suggested a role for YAP signalling in this process. scRNA-seq of mouse hepatoblasts undergoing differentiation to cholangiocytes identified *Tead1* and *Tead4* as significantly upregulated during this process[68]. The TEAD1 transcription factor motif was found to be enriched in an analysis of enhancer regions co-bound by the master regulators of liver development, HNF4α and FOXA2, in mouse hepatoblasts from E14.5 livers[69]. *Tead2* was highly expressed in embryonic liver, and expression of TEAD2 and YAP1 increased in vitro luciferase reporter expression driven by enhancers regulated by HNF4α and FOXA2, suggesting a role for YAP in regulating hepatoblast differentiation[69]. Mice with deletion of *Lats1* and *Lats2* in hepatoblasts via albumin–Cre die prior to weaning owing to lack of functional hepatocytes, as their livers consist of large numbers of immature cholangiocytes[70]. Mechanistically, this aberrantly enhanced cholangiocyte differentiation is due to increased TGFβ signalling through YAP-driven expression of *Tgfb2*. Furthermore, expression of constitutively active YAP in mouse hepatoblasts decreases *Hnf4a* expression, suggesting that YAP can function to suppress hepatocyte differentiation[59,70]. Similarly, liver-specific deletion of *Mst1* and *Mst2* (REF.[71]), *Sav1* (REF.[72]) or *Nf2* (REF.[39]) via albumin–Cre in mice results in liver overgrowth due to expansion of cells resembling immature cholangiocytes (FIG. 2e). Simultaneous deletion of *Nf2* and *Notch2* partially rescues the *Nf2* mutant phenotype, suggesting that the Notch signalling pathway might act downstream of YAP signalling during hepatoblast differentiation[73].

A 2021 study assessed the role of YAP in early hepatoblast differentiation by utilizing *Foxa3*–Cre to delete *Yap1* from hepatoblasts starting at E8.5, with complete loss of YAP from hepatoblasts by E14.5 (REF.[74]). Interestingly, early loss of *Yap1* from hepatoblasts did not impair Notch signalling or cholangiocyte specification[74]. However, newly specified cholangiocytes lacking *Yap1* failed to establish cell polarity and form luminal structures, resulting in a failure to form an intrahepatic biliary network[74]. Surprisingly, these *Foxa3*–Cre *Yap1*-knockout mice were viable and survived up to 8 months after birth despite a lack of intrahepatic bile ducts and chronic cholestasis[74]. As an adaptive mechanism, hepatocytes in *Foxa3*–Cre *Yap1*-knockout mice displayed reduced metabolic and synthetic function and increased secretion of bile acids into the bloodstream[74]. Together, these results suggest that YAP is dispensable for cholangiocyte specification but is essential to promote proper maturation of cholangiocytes and formation of the intrahepatic biliary network during liver development.

While these studies demonstrate that YAP activity must be carefully regulated during early liver development, a 2021 study demonstrated that YAP might not be required for late gestational or early postnatal hepatocyte proliferation[75]. Mice with albumin–Cre-mediated deletion of the genes encoding YAP and TAZ develop normally and do not display a reduction in liver size or hepatocyte proliferation[75,76]. However, hepatocytes from YAP-null livers display reduced viability[39], consistent with the observation that YAP promotes resistance to apoptosis[6]. Furthermore, YAP–TAZ-null livers fail to form mature bile ducts[75,76], triggering progressive development of hepatic steatosis, fibrosis and liver enlargement during adulthood[39] (FIG. 2f). Together, these studies suggest that YAP–TAZ activity has a more pronounced role in cholangiocyte rather than hepatocyte development and function.

Hippo signalling in liver homeostasis

Hippo signalling has a crucial role in maintaining adult hepatic quiescence by limiting cell proliferation, as overexpression of YAP in the liver is sufficient to induce dramatic hepatomegaly in mice and zebrafish[6,7,63]. Importantly, Hippo signalling has emerged as a master regulator of liver cell fate. Doxycycline-induced expression of YAP-S127A or acute deletion of *Nf2* in adult mouse hepatocytes triggers their dedifferentiation to cells expressing cholangiocyte markers such as SRY-box 9 (SOX9) and cytokeratin 19 (CK19)[58]. Notch signalling was identified as a crucial downstream pathway in the dedifferentiation process[58]. Cessation of YAP-S127A expression allows cells to re-differentiate into hepatocytes, highlighting the plasticity of liver cell fate[58]. YAP signalling also has major roles in cholangiocyte homeostasis. Numerous groups have described active YAP signalling in adult cholangiocytes[58,73,77,78]. One study demonstrated that YAP is required for maintenance of adult cholangiocytes, as long-term deletion of *Yap1* throughout the entire mouse liver led to bile duct paucity and development of hepatic necrosis[79]. Similarly, lack of bile ducts is observed in adult albumin–Cre *Yap1*-knockout or *Yap1/Wwtr1*-knockout mice[39,76] and mice with conditional cholangiocyte-specific *Yap1/Wwtr1* deletion[75]. YAP activity in cholangiocytes has been found to be driven by exposure to hydrophobic bile acids[56,79], and bile acid sequestration prevents loss of bile ducts in long-surviving *Yap1*-deleted mice[79], suggesting that active YAP signalling functions to prevent cell death in adult cholangiocytes (FIG. 3a). In contrast to its vital role in cholangiocytes, YAP seems to have a minimal role in hepatocytes under homeostatic conditions. Deletion of *Yap1* from adult hepatocytes results in no discernible change of phenotype in the liver[78,79], although compensatory upregulation of TAZ might confound this observation[37]. YAP seems to be minimally expressed in adult hepatocytes. In mice with knock-in of enhanced green fluorescent protein (EGFP) in the YAP target gene *Ccn2* (which encodes connective tissue growth factor (CTGF)) locus, no EGFP expression was observed in hepatocytes[58]. scRNA-seq of hepatocytes isolated from

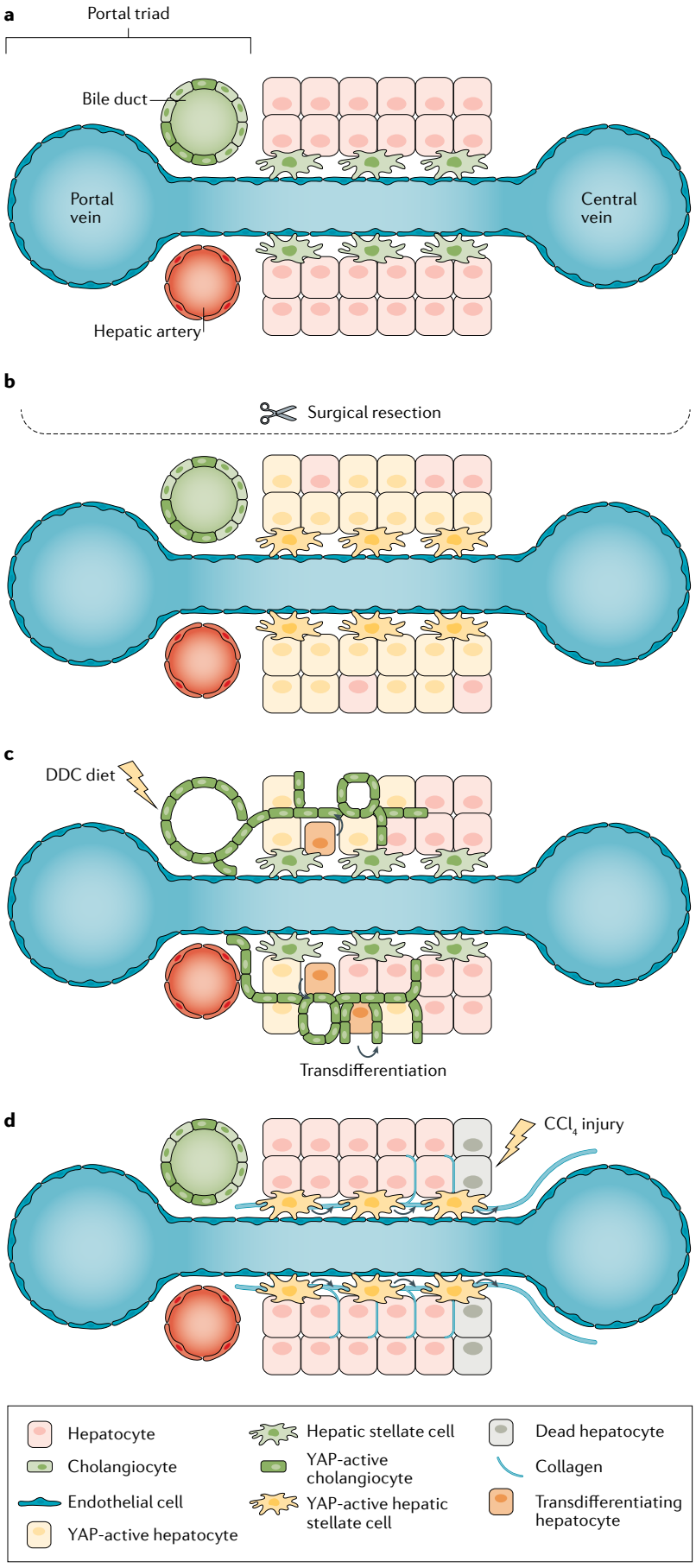


Fig. 3 | **Role of Hippo signalling in liver homeostasis and regeneration. a** | Architecture of the hepatic lobule during homeostasis. The portal triad consists of the portal vein, hepatic artery and bile ducts. Blood from the portal vein flows through the hepatic sinusoids lined by liver sinusoidal endothelial cells (LSECs) to the central vein. The sinusoids are lined by chords of hepatocytes. Hepatic stellate cells (HSCs) reside in the space of Disse (between LSECs and hepatocytes). During homeostasis yes-associated protein 1 (YAP) is active in a subset of cholangiocytes within the bile ducts. **b** | After surgical resection of liver tissue, such as during partial hepatectomy, YAP signalling is activated in HSCs and hepatocytes. **c** | Cholangiocyte injury, such as by administration of the 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet, triggers the ductular reaction. YAP activity is increased in cholangiocytes and a subset of hepatocytes. YAP activity in hepatocytes can trigger transdifferentiation to cholangiocytes. **d** | Injury induced by CCl_4 administration leads to YAP activation in HSCs and promotes collagen deposition and the development of liver fibrosis.

homeostatic mouse livers failed to detect hepatocytes with active YAP signalling gene signatures[79].

Despite seemingly negligible expression of YAP in hepatocytes, YAP has been suggested to regulate aspects of hepatic metabolism such as metabolic zonation, which is the compartmentalization of opposing metabolic processes in zones of hepatocytes based upon their localization within the hepatic lobule[65]. YAP protein staining was reported as a gradient, with nuclear localization of YAP in periportal hepatocytes and reduced staining with nuclear exclusion in pericentral hepatocytes[77]. Acute deletion of *Yap1* in adult hepatocytes led to expansion of pericentral glutamine synthetase-positive hepatocytes, while acute deletion of *Mst1* and *Mst2* reduced the number of glutamine synthetase-positive pericentral hepatocytes in mice[77]. Similarly, inducible liver-specific deletion of the F-actin capping protein CapZβ resulted in YAP hyperactivation and reduced expression of pericentral metabolic genes such as that encoding glutamine synthetase[80]. However, a study by Verboven et al. found no alterations in hepatic zonation in liver-specific *Yap1*-knockout and *Wwtr1*-knockout mice[75]. Thus, the role of YAP in hepatic zonation remains to be clearly defined.

Hippo signalling in liver regeneration

The liver possesses dramatic regenerative capacity, as both hepatocytes and cholangiocytes are capable of exiting quiescence and proliferating to restore liver mass after injury[81–83]. In extreme liver injury, cholangiocytes are capable of transdifferentiation into hepatocytes[84–87] or vice versa[88,89]. Owing to its well-known role in regulating cell proliferation, the role of Hippo signalling in liver regeneration has been extensively studied. A common acute liver injury model is acetaminophen (APAP) overdose, which induces centrilobular necrosis and subsequent hepatocyte proliferation. Mice with liver-specific hyperactive YAP signalling are protected from APAP-induced liver damage[80,90], although this might be due to decreased expression of the genes required to metabolize APAP into its cytotoxic metabolite, which display reduced expression in livers with YAP

hyperactivation[77,80]. Excitingly, pharmacological inhibition of MST1 and MST2 after exposure to APAP reduces hepatocyte death and improves overall survival[91], suggesting that YAP activation might be a novel therapeutic approach to treat APAP overdose. However, a 2021 study found that mice with deletion of *Yap1* from hepatocytes subjected to APAP overdose display equal levels of liver injury but accelerated recovery due to more rapid WNT–β-catenin-induced hepatocyte proliferation and a more pro-regenerative inflammatory response compared with control animals[92]. While this work does not necessarily disprove the idea that YAP hyperactivation is beneficial during liver regeneration from APAP-induced liver injury, it suggests that the Hippo signalling pathway has dynamic functions during liver regeneration.

One of the most common models to study hepatocyte-driven liver regeneration is the two-thirds partial hepatectomy model, where two-thirds of the liver is surgically removed, inducing hepatocyte proliferation until the liver returns to its normal size. A decrease in phosphorylated (inactive) YAP and an increase in nuclear YAP localization in hepatocytes were evident as an early event after partial hepatectomy in mice[90,93], and pharmacological inhibition of MST1 and MST2 promoted hepatocyte proliferation and enhanced liver regeneration in mice after partial hepatectomy[91]. Interestingly, YAP signalling in hepatic stellate cells (HSCs) was found to promote liver regeneration after partial hepatectomy[94]. Hedgehog signalling was identified as an upstream activator of YAP in HSCs, and inhibition of Hedgehog signalling in HSCs reduced YAP nuclear accumulation in hepatocytes after partial hepatectomy, suggesting a YAP-dependent crosstalk between HSCs and hepatocytes during liver regeneration[94] (FIG. 3b). Finally, mice with albumin–Cre-mediated deletion of the genes encoding both YAP and TAZ displayed major delays in liver regeneration after partial hepatectomy owing to decreased hepatocyte proliferation[76]. However, these results might be confounded by the fact that YAP and TAZ were deleted during embryonic liver development.

A study by Verboven et al. sought to carefully assess the cell type-specific role of YAP–TAZ in liver regeneration[75]. Mice were subjected to partial hepatectomy or acute liver injury via carbon tetrachloride (CCl_4) administration, which results in centrilobular hepatocyte death. Surprisingly, while hepatocytes displayed robust activation of YAP–TAZ signalling 48 h after CCl_4 exposure, deletion of *Yap1* and *Wwtr1* from hepatocytes did not impair liver regeneration either after partial hepatectomy or after CCl_4-induced liver injury. Instead, deletion of *Yap1* and *Wwtr1* from cholangiocytes resulted in delayed liver regeneration via cholestasis-induced delays in the recruitment of phagocytic macrophages and clearance of dead hepatocytes[75]. These results demonstrate that YAP–TAZ activation in hepatocytes is not required for liver regeneration from acute hepatic injury. This observation is not necessarily surprising, as the liver is able to compensate for the inhibition of a specific signalling pathway by activating alternative pathways during hepatocyte proliferation[95] and YAP and TAZ are not required for hepatocyte proliferation in postnatal liver development[75,76]. However, as discussed in the next section, YAP–TAZ activity is essential for models of cholangiocyte-driven liver regeneration.

Hippo signalling during chronic liver injury

YAP signalling has also been implicated in regeneration during chronic liver injury. A common feature of CLD of nearly any aetiology is the ductular reaction, or expansion of cells expressing cholangiocyte markers. Depending on the injury context, the cells of the ductular reaction can originate from proliferation of cholangiocytes or dedifferentiation of hepatocytes[96]. Hippo signalling has been identified as a crucial regulator of this process. Increased YAP activity is observed in cholangiocytes isolated from the livers of mice subjected to bile duct ligation (BDL)[79], and deletion of *Yap1* in the liver via Mx1–Cre impairs the ductular reaction, decreases hepatocyte proliferation and increases hepatic necrosis after BDL[78]. A CRISPR–Cas9 loss-of-function screen in liver biliary organoids demonstrated that YAP is essential for liver organoid growth in vitro[83]. Furthermore, mice with *Yap1* deletion in both hepatocytes and cholangiocytes placed on a 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet displayed a dramatically reduced ductular reaction owing to decreased proliferation and increased apoptosis of $CK19^+$ cells[83]. Interestingly, a study identified alternative splicing of Hippo pathway components to neonatal isoforms in hepatocytes as a mechanism of increased YAP activity and hepatocyte proliferation in response to DDC diet-induced liver injury in mice[97]. Camargo and colleagues have shown via scRNA-seq increased numbers of YAP-active cholangiocytes as well as increased YAP activity in hepatocytes isolated from DDC diet-fed animals[79] (FIG. 3c). Furthermore, deletion of *Yap1* specifically in hepatocytes reduced the extent of the ductular reaction in DDC diet-fed animals, implying that hepatocyte dedifferentiation is a major source of ductular reaction cells in DDC diet-induced liver injury[79]. A study by Li et al. identified *Arid1a*, which encodes a component of the ATP-dependent SWI/SNF chromatin remodelling complex, as a key player in this process[98]. Deletion of *Arid1a* specifically from hepatocytes reduced the number of $HNF4\alpha^+SOX9^+$ hepatocytes in the livers of animals fed a DDC diet for 2 weeks. Furthermore, chromatin accessibility studies revealed that a substantial number of genes that were enriched in DDC-induced $HNF4\alpha^+SOX9^+$ hepatocytes were co-bound by YAP and ARID1A. YAP binding to target sites and target gene expression were significantly reduced in *Arid1a*-null hepatocytes isolated from DDC diet-fed animals, leading the researchers to conclude that ARID1A facilitates YAP-mediated transcription during liver regeneration[98]. However, a separate study performed by Chang et al. found exactly the opposite phenotype[99]. Mice with hepatocyte-specific deletion of *Arid1a* fed a DDC diet for 6 weeks displayed an increased ductular reaction, and this phenotype was abrogated in *Arid1a*/*Yap1*/*Wwtr1* triple-mutant livers. Furthermore, hepatocyte-specific deletion of both *Nf2* and *Arid1a* triggered dramatic liver overgrowth, strong induction of YAP–TAZ transcriptional activity, and development of liver cancer[99]. ARID1A seems to be dispensable for YAP regulation during homeostasis, as both groups

found that *Arid1a*-deleted livers seemed to be normal under resting conditions[98,99]. A possible explanation for these conflicting observations is the different duration of DDC diet, which might have influenced the extent of liver injury and the ductular reaction. More research is necessary to clarify the role of ARID1A in the regulation of YAP activity during liver regeneration.

Hippo signalling has also been implicated in the development of liver fibrosis. During acute liver injury, activation of HSCs (widely considered to be the main source of liver myofibroblasts) and deposition of ECM is an intrinsic component of the wound-healing response[3]. However, during CLD, iterative cycles of hepatic damage and repair trigger continuous activation of HSCs and eventually exhaust the regenerative potential of the liver, leading to massive deposition of ECM and loss of organ function as fibrosis progresses to cirrhosis[3]. A common mouse model used to study fibrosis progression in mice is chronic CCl_4 administration (FIG. 3d). One study found that expression of YAP-S127A in hepatocytes of mice exposed to CCl_4 promoted expression of CYR61 (also known as CCN1), a macrophage chemoattractant, and increased inflammation, myofibroblast expansion and fibrosis. Furthermore, deletion of *Yap1* and *Wwtr1* from hepatocytes reduced CCl_4-induced liver fibrosis[100]. A separate group identified increased expression of TAZ in hepatocytes in samples from patients with fibrosis due to nonalcoholic steatohepatitis (NASH)[61], a condition characterized by accumulation of fat in the liver accompanied by inflammation, hepatocyte death and fibrosis. These findings were confirmed in multiple mouse models of NASH, in which elevated expression of TAZ in hepatocytes increased expression of the TAZ target gene encoding Indian hedgehog, which increased activation of HSCs and promoted fibrosis[61]. Interestingly, a separate group found that YAP activation was protective against fibrosis in a mouse model of ischaemia–reperfusion injury in a NRF2-dependent manner. Mechanistically, YAP activation reduces oxidative stress, apoptosis, innate immune activation and HSC activation[101]. The discrepancy between these studies might be explained by the fact that YAP activation prior to onset of ischaemia–reperfusion injury reduces overall hepatocyte death[101] and therefore reduces the subsequent injury response. These results demonstrate that, depending on the liver injury context, YAP–TAZ activation can be either pro-fibrotic or anti-fibrotic.

YAP, but not TAZ[61], has been identified as a driver of HSC activation. Nuclear YAP staining is not detected in HSCs from healthy livers but is evident in the fibrotic livers of both humans and mice[102]. Isolated primary HSCs are known to undergo transdifferentiation to myofibroblasts when grown under conventional 2D culture conditions[102]. Treatment with the YAP inhibitor verteporfin prevented upregulation of *Acta2* and *Col1a1* in cultured mouse HSCs and reduced fibrogenesis after chronic CCl_4 treatment in vivo, suggesting that YAP signalling is required for HSC activation[102,103]. Mechanistically, activation of HSCs is partly driven by the stiffness of the surrounding environment, as HSCs grown in 3D spheroids in vitro remain quiescent and do not upregulate expression of YAP target genes[102]. As described earlier in this Review, YAP is a well-known mechanotransducer that is activated in cells grown on high-stiffness substrates[29]. Consistent with these reports, loss of integrin β1 in cultured HSCs inhibits induction of fibrotic markers and prevents increases in YAP expression and nuclear localization[103].

In addition to HSCs, Hippo signalling in other non-parenchymal cell (NPC) types has been found to influence CLD. One study found that YAP activation in Kupffer cells, the resident liver macrophages, has a role in promoting NASH progression[104]. Mice fed a high-fat diet (HFD) for 12 weeks displayed increased YAP expression and nuclear accumulation in Kupffer cells. Macrophage and monocyte-specific YAP deletion reduced pro-inflammatory cytokine expression and lobular inflammation without affecting steatosis in HFD-fed mice, and expression of constitutively active YAP in primary Kupffer cells increased pro-inflammatory cytokine expression[104]. A 2021 study found increased YAP levels in liver macrophages in both human cirrhotic liver samples and diet-induced or chemical-induced mouse liver fibrosis models[105]. Interestingly, loss of *Yap1* specifically in myeloid cells in mice promoted type I interferon signalling in liver macrophages, which in turn suppressed CTGF expression in liver endothelial cells and reduced liver fibrosis[105]. An understanding of the functions of YAP in liver sinusoidal endothelial cells (LSECs) is only just beginning to emerge. YAP has been reported to promote vessel maintenance in response to laminar shear stress in zebrafish endothelial cells[106]. A study by Zhang et al. found that YAP stabilized hypoxia-inducible factor 1α levels in LSECs, which promoted angiogenesis in CCl_4-induced liver fibrosis[107]. Thus, YAP activity in NPCs might facilitate disease progression, although more research is necessary to fully elucidate the role of Hippo signalling in these cell types.

Hippo signalling in liver cancer

Liver cancer is the sixth leading cause of cancer worldwide and has a 5-year survival rate of only 18%[1]. The lack of effective treatments for liver cancer has prompted intense investigation into the molecular mechanisms promoting tumorigenesis that represent potentially druggable targets. To this end, the Hippo signalling pathway has been extensively studied, as chronic overexpression of YAP[6] or loss of *Mst1* and *Mst2* (REFS[71,108,109]), *Nf2* (REF.[39]) or *Sav1* (REF.[109]) in the mouse liver triggers the eventual development of liver cancers including hepatocellular carcinoma (HCC) and cholangiocarcinoma.

Hippo signalling in hepatocellular carcinoma. HCC is the most common primary liver cancer, and nuclear accumulation of YAP is frequently observed in human HCC samples[6,17,110,111]. Activation of YAP is associated with more aggressive subtypes of HCC[77,112], including a concordance between a silenced Hippo pathway gene signature and a hepatic stem cell gene signature[113]. Patients with HCC displaying the silenced Hippo pathway gene signature exhibited significantly reduced overall survival compared with patients with HCC lacking this signature ($P = 9.5 \times 10^{-5}$)[113], demonstrating that YAP activation is an independent prognostic marker

in HCC[112,113]. However, mutation of components of the Hippo pathway is rarely observed in liver cancer[114], although focal amplification of human chromosome 11q22, which contains the *YAP1* gene, has been reported as a low-frequency event in HCC[115]. The lack of mutations suggests that inhibition of the Hippo pathway or activation of YAP occurs through crosstalk with other oncogenic signalling pathways during tumorigenesis.

The upstream mechanisms that activate YAP in liver cancer have not been fully elucidated, but several likely candidates have emerged (FIG. 4a). Bile acid overload has been shown to promote liver tumorigenesis

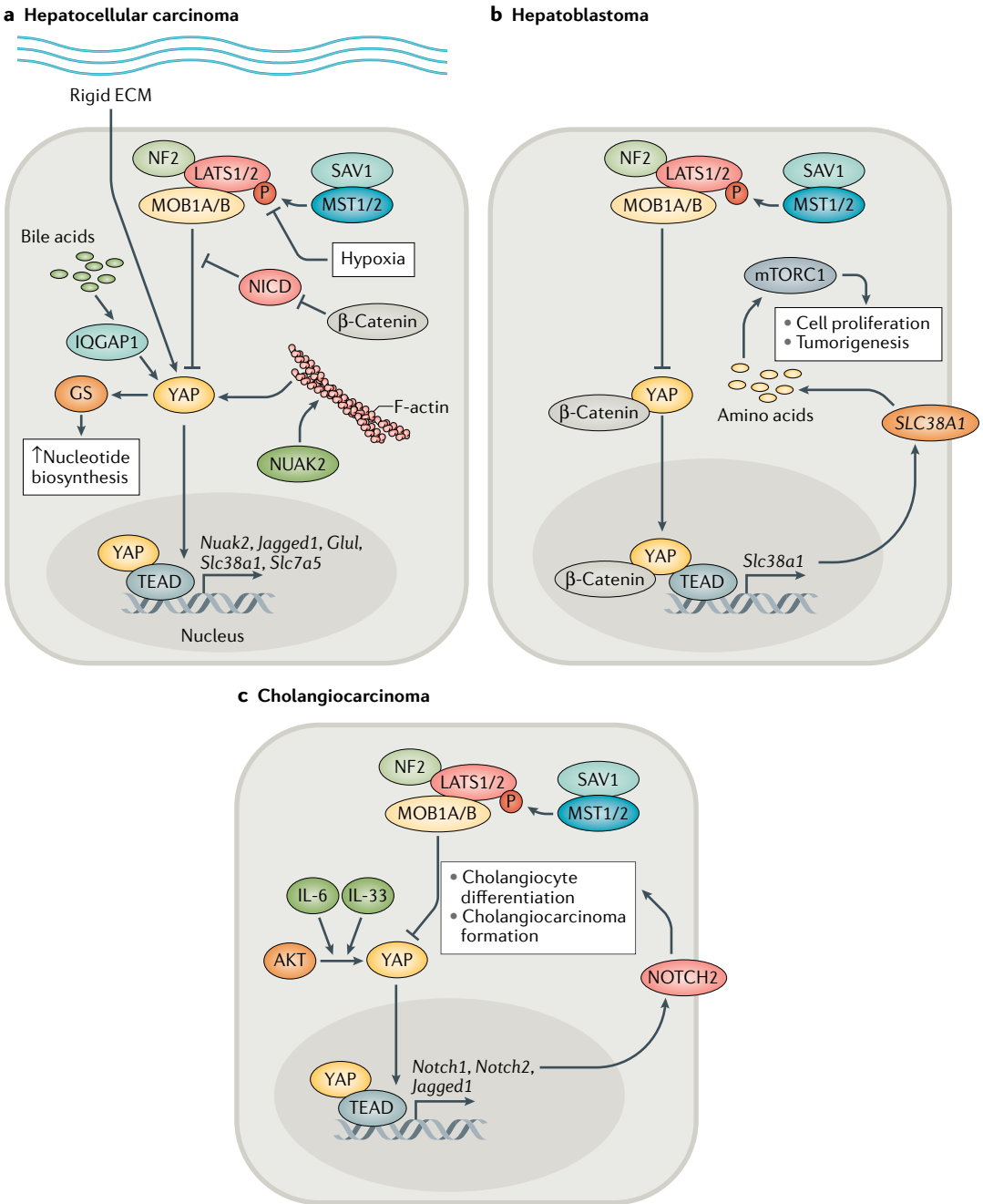


Fig. 4 | **Role of Hippo signalling in liver cancer. a** | In hepatocellular carcinoma, factors such as rigid extracellular matrix (ECM), bile acid exposure, hypoxia and increased actomyosin tension can trigger yes-associated protein 1 (YAP) activation. YAP can promote tumorigenesis through increased nucleotide biosynthesis, enhanced Notch signalling, and expression of amino acid transporters. β-Catenin inhibits YAP activity through inhibition of Notch signalling. **b** | In hepatoblastoma, β-catenin and YAP cooperate to promote tumorigenesis through expression of the amino acid transporter SLC38A1. Increased accumulation of amino acids activates mechanistic target of rapamycin complex 1 (mTORC1) signalling to promote tumorigenesis. **c** | In cholangiocarcinoma, AKT and YAP can cooperate to enhance Notch signalling, which in turn promotes cholangiocyte differentiation and tumorigenesis. The inflammatory cytokines IL-6 and IL-33 promote this process. F-actin, actin filaments; GS, glutamine synthetase; IQGAP1, Ras GTPase-activating-like protein 1; MOB1A/B, MOB kinase activator 1A/B; NICD, Notch intracellular domain; NUAK2, NUAK family SNF1-like kinase 2; SAV1, salvador homologue 1.

in mice[56,116,117], and bile acid-mediated activation of IQGAP1 has been shown to promote YAP activity[56]. Importantly, expression of YAP and IQGAP1 was minimal in healthy livers but was detected in cholestatic diseased livers and in HCC[56], suggesting that bile acids might promote YAP activation as a mechanism of tumorigenesis. Interestingly, a 2021 study identified glycogen accumulation as a driver of liver tumorigenesis[118]. In both human and mouse HCC samples, early malignant lesions and small tumour nodules displayed high glycogen content. Condensed glycogen underwent liquid–liquid phase separation, and the glycogen-binding protein laforin was found to bind strongly to MST1 or MST2, resulting in their sequestration in glycogen droplets, leading to activation and nuclear translocation of YAP in multiple cultured human cell lines, including HepG2 cells, as well as in mouse hepatocytes in vivo. Importantly, increased glycogen accumulation led to increased tumour incidence in a YAP-dependent manner in multiple genetic mouse models of liver tumorigenesis, suggesting that glycogen droplets may have an important role in promoting tumour initiation through activation of YAP[118]. Tyrosine kinase receptors are major oncogenic pathways in liver cancer and have also been shown to interact with Hippo signalling. EGFR signals through PI3K–PDK1 to activate YAP in HCC, and treatment of human HCC cell lines simultaneously with EGFR and YAP inhibitors synergistically induce cytotoxicity[119]. Mice with liver-specific deletion of *Sav1* and the gene encoding phosphatase and tensin homologue (PTEN) develop fatty liver, inflammation and liver tumours by 15 weeks of age. Mechanistically, YAP–TAZ increases expression of insulin receptor substrate 2, which in turn activates AKT and promotes tumorigenesis[64]. These studies highlight the role of crosstalk between YAP–TAZ and other oncogenic signalling pathways in the development of HCC.

As discussed earlier, YAP is a mechanotransducer and is activated in cells grown on stiff substrates[30,31]. As cirrhosis is a major risk factor for HCC[3], this raises the question of whether increased liver stiffness during cirrhosis activates YAP signalling and potentiates tumorigenesis. One analysis of tumour samples from patients with liver cancer in The Cancer Genome Atlas (TCGA) found that increased mRNA expression of the ECM proteoglycan agrin is associated with reduced overall survival ($P < 0.00016$)[120]. Increased ECM stiffness activates agrin, which in turn activates YAP activity to promote oncogenesis in liver cancer cell lines[120]. Furthermore, HCC cell lines grown on substrates mimicking the stiffness of cirrhotic livers display increased resistance to sorafenib in a YAP-dependent manner[121], suggesting that the mechanotransduction functions of YAP might have a role in promoting HCC. Multidrug resistance is common in HCC, and intratumour hypoxia has been shown to promote HCC resistance to sorafenib[122]. Hypoxia has been shown to trigger YAP activation through inhibition of LATS2 (REF.[123]), which led to increased sorafenib resistance in HCC cells[124]. Additionally, in oncogene-induced mouse autochthonous HCC models, high YAP–TAZ activity in cancer cells promoted activation of tumour stromal cells and impaired penetration of verteporfin into the tumour[125], suggesting YAP-dependent impaired drug trafficking as another mechanism of drug resistance in HCC.

YAP transcriptional activity in HCC has been shown to promote tumorigenesis through multiple mechanisms. In a 2018 study, Camargo and colleagues identified the kinase NUAK2 as a direct transcriptional target of YAP. NUAK2 activity triggered actin polymerization and increased actomyosin tension to further promote YAP activity, and NUAK2 inhibition reduced tumorigenesis in a YAP-driven liver cancer mouse model[60]. A separate group found that YAP activity promoted cell viability, migration, and invasion in HCC cell lines through induction of *JAG1* and activation of Notch signalling in a TEAD4-dependent manner. Furthermore, nuclear expression of YAP, Jagged1 and the Notch intracellular domain (NICD) are associated with poor prognosis in patients with HCC[110]. Interestingly, YAP induction of *JAG1* is regulated by MST1 and MST2 but not by β-catenin[110,126]. Activating mutations in β-catenin (encoded by *CTNNB1*) are present in approximately 30% of human HCC samples, but expression of YAP is negatively correlated with expression of β-catenin in HCC tumours[77,127], suggesting antagonism between the two pathways in the context of HCC. Indeed, liver-specific deletion of *Mst1* and *Mst2* in mice increases tumorigenesis through YAP–TAZ activation, which forms a positive feedback loop with Notch signalling as increased NICD expression prevents TAZ protein degradation and further promotes YAP–TAZ activity. Co-deletion of *Ctnnb1* and *Mst1*/*Mst2* dramatically increases tumorigenesis, as increased β-catenin activity promotes expression of dimerization partner 1, which in turn promotes NICD degradation and inhibition of Notch signalling[126].

Hippo signalling in hepatoblastoma. Paradoxically, while YAP and β-catenin seem to have an antagonistic relationship in HCC, they seem to cooperate in the promotion of hepatoblastoma, the most common paediatric liver cancer[62]. Nuclear accumulation of both YAP and β-catenin was observed in approximately 80% of tumours in a study of 94 samples from patients with hepatoblastoma, and sleeping beauty transposon system hydrodynamic tail veil injection (SB-HDTVI)-mediated delivery of YAP-S127A and constitutively active β-catenin into hepatocytes led to the rapid development of hepatoblastoma in mice[127]. Later studies found that in hepatoblastoma cells YAP–TAZ promoted expression of the amino acid transporter SLC38A1, triggering activation of mTORC1 and tumorigenesis[62] (FIG. 4b). Interestingly, YAP–TAZ-driven upregulation of amino acid transporters[128] or enhanced nucleotide biosynthesis[63] triggering downstream mTORC1 activation has also been identified in HCC, suggesting crosstalk between the YAP and mTOR signalling pathways as a common driver of liver carcinogenesis. Importantly, in a 2020 study, silencing of YAP-S127A in the YAP-S127A/constitutively-active β-catenin-driven hepatoblastoma mouse model resulted in dramatic tumour regression, driven by apoptosis in a subset of tumour cells and partially restored hepatocyte differentiation in surviving tumour cells[129]. These results

suggest that targeting YAP might be an effective therapy for hepatoblastoma.

Hippo signalling in cholangiocarcinoma. YAP has also been hypothesized to be an oncogenic driver of cholangiocarcinoma, the second most common type of primary liver cancer[1]. Nuclear YAP accumulation is observed in 85–98% of human cholangiocarcinoma tissue samples[111,130,131] and is associated with a poor prognosis[130,132]. YAP activation is also associated with increased cholangiocarcinoma proliferation[130,131], invasion[130], angiogenesis[131], chromosomal instability[133] and chemoresistance[130,131]. Dual SB-HDTVI-mediated expression of NICD and AKT in hepatocytes[134,135] or YAP-S127A and oncogenic AKT in hepatocytes[136] or cholangiocytes[137] led to rapid cholangiocarcinoma formation (FIG. 4c). Activation of mTORC1 was found to be a downstream target of YAP, as deletion of *Rptor* (encoding Raptor, a major component of the mTORC1 signalling complex) suppressed tumour development in a NICD/YAP-S127A mouse model of cholangiocarcinoma[138]. FGFR signalling was found to upregulate YAP expression in cholangiocarcinoma cell lines, and treatment with a pan-FGFR inhibitor reduced tumour burden in a YAP–AKT-driven cholangiocarcinoma mouse model[47]. Mechanotransduction has also been identified as an important regulator of cholangiocarcinoma development[139]. FAK was found to directly phosphorylate YAP-Y357 and promote its nuclear localization in cholangiocarcinoma cell lines, and deletion of *Ptk2* (the gene encoding FAK) suppressed tumour development in the AKT–YAP-S127A mouse model of cholangiocarcinoma. Furthermore, overexpression of oncogenic AKT and FAK in mouse hepatocytes led to rapid cholangiocarcinoma development[139]. Importantly, a study of 50 human cholangiocarcinoma samples demonstrated that higher mRNA expression of *PTK2* correlates with reduced patient survival ($P < 0.0001$)[139], suggesting that mechanotransduction might be an important driver of cholangiocarcinogenesis in humans.

Deletion of *Yap1* and *Wwtr1* from established cholangiocarcinoma tumours in mice dramatically reduces tumour burden[135], suggesting an important role for YAP in driving cholangiocarcinoma. In addition to the tumour cells themselves, activation of YAP in peritumoural hepatocytes has been observed in both HCC and cholangiocarcinoma[135]. Surprisingly, deletion of *Yap* and *Wwtr1* specifically in peritumoural hepatocytes leads to increased tumour burden. Activation of YAP in peritumoural hepatocytes reduced tumour burden in a NICD–AKT-driven cholangiocarcinoma mouse model as well as in models of HCC and melanoma liver metastasis[135]. However, deletion of *Yap* and *Wwtr1* in both tumour cells and peritumoural hepatocytes caused no change in tumour burden. Although the exact mechanism remains unclear, increased cell death was detected in tumour cells surrounded by YAP-active hepatocytes, leading the researchers to speculate that these cells display increased fitness during cell competition with tumour cells[135]. More studies are necessary to elucidate the mechanisms of YAP activation in peritumoural hepatocytes on the suppression of liver cancer.

Hippo signalling and cancer immunity. Hippo signalling has also gained attention for its emerging role in cancer immunity. The field of cancer immunotherapy has made great advances in treatment strategies, such as anti-PD1/PDL1 therapy to reactivate T cells to promote tumour cell killing, although resistance or relapse remains a major clinical problem[140]. YAP and TAZ have been shown to induce expression of PDL1 in human melanoma, lung and breast cancer cell lines[141–144], suggesting a role for YAP–TAZ in suppressing anti-tumour immune responses. However, these results could not be replicated in murine cell lines[143], suggesting species-specific differences in the regulation of PDL1. Despite the knowledge that PDL1 expression in HCC is correlated with a poor prognosis[145], the relationship between YAP–TAZ and PDL1 in the context of liver cancer remains to be investigated. In contrast to these studies, YAP–TAZ expression in tumour cells has also been found to promote adaptive immunity anti-cancer responses. Deletion of *LATS1* and *LATS2* from melanoma, head and neck squamous cell carcinoma and breast cancer cell lines promoted tumour growth in vitro but dramatically inhibited tumour growth in vivo during subcutaneous tumour cell transplantation into immunocompetent syngeneic mice[146]. It was found that deletion of *LATS1* and *LATS2* increases production of extracellular vesicles rich in nucleic acids, which stimulates the type I interferon response and enhanced anti-tumour immunity[146]. In the context of the liver, YAP–TAZ activation in hepatocytes have consistently been shown to promote inflammation. Deletion of *Lats1*/*Lats2* or *Mst1*/*Mst2* or YAP activation in hepatocytes rapidly triggers immune cell recruitment[59,100,147,148]. Oncogenic YAP activation in hepatocytes induced *Ccl2* expression, which promoted macrophage recruitment, immune evasion and tumorigenesis in mice[147,148]. These results suggest that the inflammation triggered by YAP activation is a key mechanism of tumorigenesis. Interestingly, a 2021 study demonstrated that pro-inflammatory cytokines released during chronic inflammation in a murine NASH model suppresses expression in hepatocytes of epithelial splicing regulatory protein 2 (ESRP2), which is responsible for the splicing of the mature form of *Nf2* (REF.[149]). Loss of ESRP2 results in accumulation of the less functional fetal isoform of NF2, leading to increased activation of YAP–TAZ[149], suggesting a potential positive feedback loop between chronic inflammation and YAP–TAZ activity. Although the role of YAP-induced inflammation in tumour maintenance and progression is less well defined, it seems logical that targeting YAP–TAZ could potentially synergize with immunotherapy, although the efficacy of this approach remains to be determined.

Pharmacological targeting of the Hippo signalling pathway

Owing to its putative roles as an oncogene and in promoting the progression of CLD, there has been great interest in developing small-molecule inhibitors of YAP activity (FIG. 5a). Classically, verteporfin was utilized as an inhibitor of YAP–TEAD interactions[150]. However, work in the past 5 years has called into question this mechanism of action, precluding the use of verteporfin as

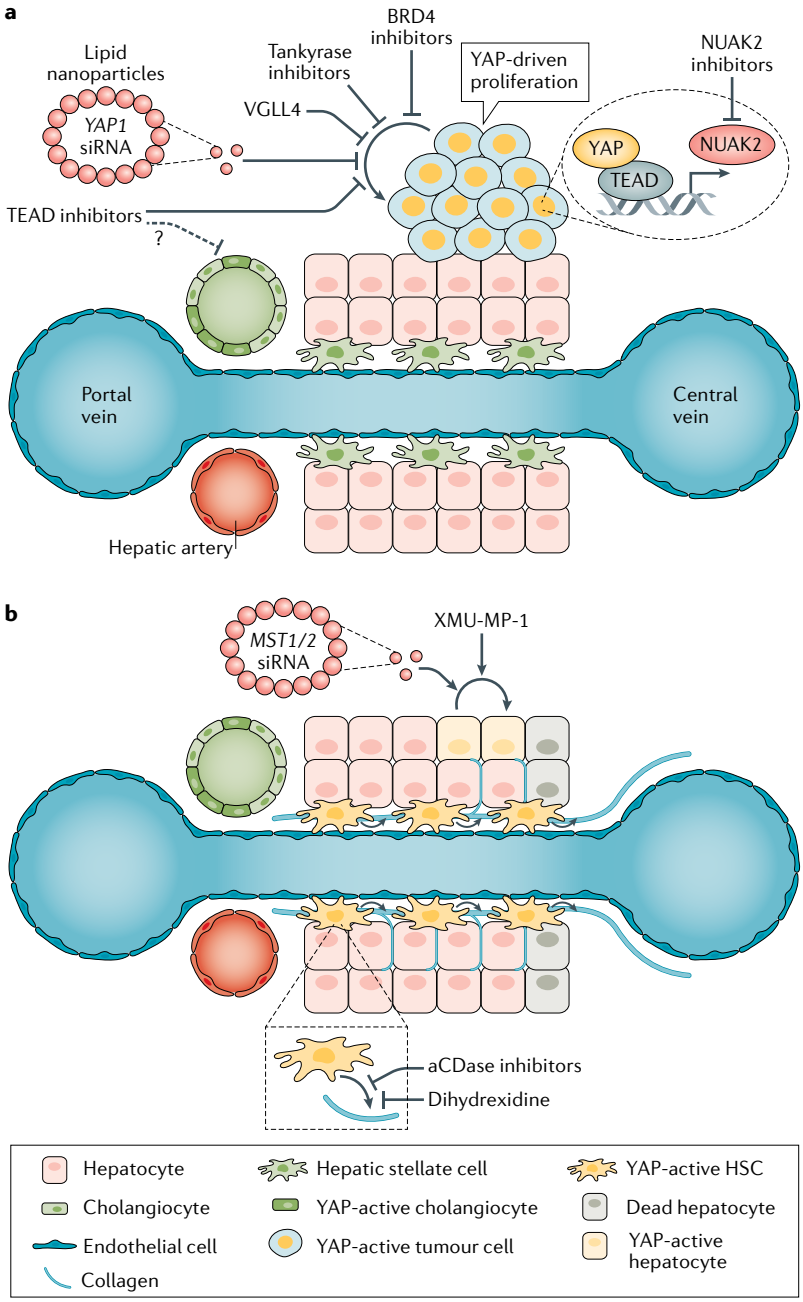


Fig. 5 | **Strategies for targeting the Hippo signalling pathway. a** | Strategies to suppress liver cancer by inhibiting yes-associated protein 1 (YAP) activity in tumour cells. **b** | Strategies to promote liver regeneration by augmenting YAP activity in hepatocytes and to inhibit fibrosis progression by specifically inhibiting YAP activity in hepatic stellate cells (HSCs). aCDase, ceramide-metabolizing enzyme; BRD4, bromodomain-containing protein 4; NUAK2, NUAK family SNF1-like kinase 2; siRNA, small interfering RNA; VGLL4, vestigial-like family member 4; XMU-MP-1, MST1 and MST2 inhibitor.

a specific inhibitor of YAP activity[151]. Much research has been devoted to developing a specific inhibitor of the YAP–TEAD protein–protein interaction, although this is difficult owing to its unusually large interface and lack of a defined binding pocket[152]. One strategy includes targeting the TEAD palmitate-binding pocket, resulting in allosteric inhibition of the YAP–TEAD interaction[153,154]. Similarly, a covalent TEAD inhibitor promoted apoptosis in non-small-cell lung cancer cells with YAP-dependent resistance to EGFR–MEK inhibition[155]. Another strategy involves exploitation of endogenous proteins that also bind to TEAD or manipulation of regulators of YAP activity. One group demonstrated that vestigial-like family member 4 (VGLL4) competes with YAP for binding to TEADs, and a VGLL4-mimicking peptide inhibits YAP activity and reduces gastric cancer growth both in vitro and in vivo[156]. Inhibitors targeting positive regulators of YAP activity such as tankyrases[157,158] or transcriptional regulators such as bromodomain-containing protein 4 (BRD4), which mediates YAP–TAZ-driven transcription[159], have also been considered, although these inhibitors lack specificity to the Hippo signalling pathway. Another approach has been to target disease-specific pathway components rather than YAP–TEAD directly. For example, Camargo and colleagues identified NUAK2 as a critical downstream target of YAP during liver tumorigenesis, and pharmacological inhibition of NUAK2 suppresses YAP-driven tumour growth in vivo[60]. This type of approach might reduce potential off-target effects rather than directly targeting components of such a pleiotropic signalling pathway.

Despite the abundant evidence of the oncogenic role of YAP–TAZ in liver cancer, strategies for targeting the Hippo pathway must be designed with great care. For example, inhibiting YAP–TAZ in homeostatic hepatocytes would probably be well tolerated, whereas inhibition in cholangiocytes would most likely have deleterious effects on liver function[75,79]. Furthermore, Moya et al. demonstrated that genetic deletion of YAP–TAZ specifically in tumour cells reduced tumour burden in a cholangiocarcinoma mouse model, but YAP–TAZ activity in peritumoural hepatocytes suppressed tumour growth[135]. These results suggest that indiscriminate YAP–TAZ inhibition might be of limited efficacy. An exciting strategy to circumvent these problems is to target YAP–TAZ inhibition to specific cell types. For example, small interfering RNA (siRNA) against *Yap1* or components of the Hippo pathway can be formulated into lipid nanoparticles that target hepatocytes[160]. However, it will be important to develop technology to deliver siRNA or small molecules specifically to tumour cells to promote the greatest efficacy and minimize off-target effects.

In addition to cancer, there is also interest in inhibiting YAP–TAZ as an anti-fibrotic therapy (FIG. 5b). Again, cell type-specific targeting has been shown to be important. Despite evidence of YAP activity in fibroblasts promoting fibrosis progression, general YAP–TAZ RNA interference in a mouse model of pulmonary fibrosis actually increased lung injury and fibrosis[161]. Thus, research has focused on methods to inhibit YAP specifically in fibroblasts. As YAP activity is known to be regulated by GPCR signalling[43], one group sought to identify whether fibroblast-specific GPCR expression could serve as the basis for novel anti-fibrotic therapies. Dihydrexidine (DHX), a selective agonist of the $G\alpha_s$-coupled dopamine D1 and D5 receptors, inhibited YAP nuclear accumulation in a panel of fibroblasts including HSCs. Excitingly, in the BDL-induced liver fibrosis mouse model, treatment with DHX reduced histological staining for collagen and α-smooth muscle

actin[161]. Similarly, antagonism of the dopamine receptor D2 inhibited YAP activity in macrophages and reduced liver fibrosis in a minipig NASH model[105]. A separate group found that the sphingolipid ceramide promoted HSC inactivation through increased YAP–TAZ proteasomal degradation. Targeting the enzyme that metabolizes ceramide, aCDase, which is expressed in activated HSCs and minimally in other liver cell types, reduced fibrosis in both CCl_4 and dietary NASH mouse models of fibrosis[162]. These results suggest that clever targeting of YAP activity specifically in HSCs might hold promise as a therapy to prevent fibrosis progression.

In addition to developing inhibitors, there is also interest in developing drugs that promote YAP–TAZ activity to augment liver regeneration (FIG. 5b). Indeed, the MST1 and MST2 inhibitor XMU-MP-1 was well tolerated in mice and significantly enhanced liver regeneration after APAP-induced liver injury or after partial hepatectomy[91]. Furthermore, siRNA-mediated knockdown of MST1 and MST2 improved liver regeneration after partial hepatectomy in aged mice[93]. Importantly, these strategies involve transient inactivation of MST1 and MST2, reducing the likelihood of oncogenesis. XMU-MP-1 treatment after repeated CCl_4 exposure or BDL reduced fibrosis compared with vehicle treatment[91], suggesting it might also have therapeutic implications for CLD. However, one study demonstrated that hepatocyte-specific deletion of YAP–TAZ reduces liver fibrosis upon exposure to CCl_4 by reducing inflammation and myofibroblast expansion[100]. A potential explanation for these contradictory results is that XMU-MP-1 was administered after cessation of CCl_4 treatment (during the liver regeneration phase), while in the study by Mooring et al. YAP–TAZ was deleted from hepatocytes prior to exposure to CCl_4 (REF.[100]). It is thus important to determine whether XMU-MP-1 treatment during ongoing liver injury promotes or inhibits fibrosis development. Nonetheless, activation of YAP–TAZ as a strategy holds promise for regenerative medicine.

Conclusions and future directions

The Hippo signalling pathway has complex, cell type-specific and context-specific roles in many aspects of hepatic function including development, regeneration and carcinogenesis. While incredible progress has been made in unravelling the complex networks of signalling pathways that interact with the Hippo pathway, much remains to be learned. For example, many studies have examined perturbation of either YAP or TAZ alone, although a growing body of evidence suggests that these proteins each have non-redundant functions[22,61,126]. Additionally, much of the research studying the effect of perturbations of Hippo signalling in a disease context have focused on transgenic animals with either deletion of Hippo signalling components or expression of constitutively active forms of YAP. While these studies have yielded useful insights, they do not enable the study of Hippo signalling in its native context or provide information on the upstream inputs that trigger pathological inactivation of the Hippo pathway. This is especially important in the context of CLD, as identification of the early triggers of pathological Hippo signalling perturbations might yield novel therapeutic strategies to prevent progression of CLD to cirrhosis and liver cancer.

An unexpected finding from studies within the past 3 years is the dispensable role of YAP–TAZ activity in hepatocyte proliferation during development and acute regeneration[75,76], which is seemingly at odds with the long-held belief that Hippo signalling is a master regulator of organ size. However, these facts can be reconciled if one adopts the view that Hippo signalling regulates organ size by preventing aberrant YAP–TAZ activity, rather than YAP–TAZ activity being essential for liver growth. YAP and TAZ are potent inducers of cell proliferation if unrestrained by the Hippo pathway, as evidenced by the role of Hippo inactivation in the development of liver cancer[39,71,108,109]. Although non-essential for hepatocyte proliferation, YAP–TAZ can increase the fitness of hepatocytes, as YAP-null hepatocytes are more prone to apoptosis[6] and YAP–TAZ activity in peritumoural hepatocytes inhibits tumour progression[135]. Furthermore, research has demonstrated that YAP–TAZ activity is essential for normal cholangiocyte function[75,79,83] and promotion of a ductular reaction after liver injury[83], underscoring the key role of Hippo signalling in liver biology.

Undoubtedly one of the most exciting research areas is development of anti-YAP–TAZ therapies for liver cancer. To this end, future studies should prioritize the ability to inhibit YAP–TAZ specifically in tumour cells. Drugs such as BRD4 inhibitors show promise, as they can preferentially inhibit YAP–TAZ transcriptional activity in cancer cells[159]. Tailored drug delivery, such as through lipid nanoparticles[160], is another attractive strategy. Further research into the role of YAP–TAZ in liver cancer immunity is also warranted. As YAP and TAZ have been shown to regulate expression of PDL1 in multiple cancer types[141–144], and PDL1 expression in HCC is correlated with a poor prognosis[145], the relationship between YAP–TAZ and PDL1 in liver cancer is an obvious area for future investigation.

Targeting YAP–TAZ activity to promote liver regeneration is another exciting field of study. To pursue these goals, a detailed understanding of disease mechanisms is necessary to identify conditions for which such therapies will be effective. The roles of YAP–TAZ activity in cell types such as endothelial cells and resident immune cells are under-studied and might have important implications. For example, non-specific targeting of YAP–TAZ in a mouse model of pulmonary fibrosis increased lung injury owing to putative detrimental effects on endothelial cells[161]. Thus, further study of the role of YAP–TAZ in NPCs in CLD is warranted, and application of technologies such as scRNA-seq to samples of diseased human liver will prove invaluable in this regard. Timing of therapeutic delivery is also critically important, as suppression of YAP–TAZ might prove beneficial during certain types of ongoing injury[100] but detrimental during liver regeneration[83,91]. Similarly, inactivation of Hippo signalling to promote liver regeneration must be tightly controlled to prevent tumorigenesis.

Finally, YAP has been identified as a master regulator of liver cell fate and an essential regulator of

hepatocyte-to-cholangiocyte transdifferentiation during liver injury[58,79,83], but the mechanisms underlying this process remain poorly defined. Further research into this topic will probably uncover key transcriptional or epigenetic mechanisms that govern hepatocyte-to-cholangiocyte transdifferentiation. This knowledge might prove useful in the effort to maintain primary hepatocytes in culture[34] or to promote induced pluripotent stem cell differentiation to mature hepatocytes, which could have implications for the in vitro study of hepatocyte drug metabolism and the ability to grow mature hepatocytes for transplantation purposes. Undoubtedly, future research will continue to uncover the roles of Hippo signalling in hepatic biology and lead to the development of therapies to combat a wide variety of liver diseases.

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F.D.C. made a substantial contribution to discussion of content, and reviewed/edited the manuscript before submission. J.O.R. researched data for the article, made a substantial contribution to discussion of content, wrote the article, and reviewed/edited the manuscript before submission.

Competing interests
F.D.C. holds equity interests and is on the scientific advisory board of Fog Therapeutics. F.D.C. has applied for patents related to inhibition of YAP/TEAD function.

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